3972

Phytochemistry, 1976, Vol. 15, pp. 1963–1965. Pergamon Press. Printed in England.

STRUCTURE OF A SECOISOLARICIRESINOL DIESTER FROM *SALVIA PLEBEIA* SEED

RICHARD G. POWELL and RONALD D. PLATTNER

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604, U.S.A.

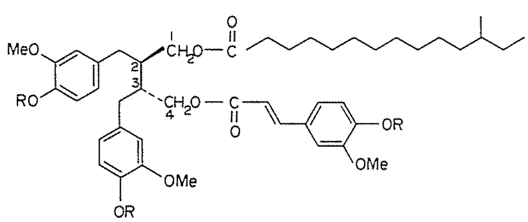
(*Revised received* 28 *June* 1976)

Key Word Index—*Salvia plebeia*; Labiatae; lignan diester; ferulic acid; *anteiso* fatty acid; (+)-12 L-methyltetradecanoic acid; secoisolariciresinol.

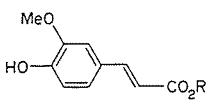
Abstract—The structure of a unique lignan diester from *Salvia plebeia* seed has been determined. Hydrolysis of the diester yields ferulic acid, (+)-12 L-methyltetradecanoic acid and secoisolariciresinol.

INTRODUCTION

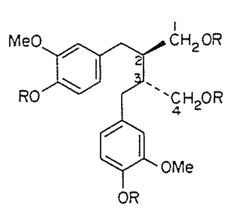
This report details the characterization of a unique lignan diester (**1a**) present in *Salvia plebeia* R. Br. seed and derived from secoisolariciresinol. Secoisolariciresinol (**4a**) is known as a heartwood constituent in several genera including *Podocarpus*, *Larix*, *Fitzroya* and *Taxus* [1–4]. The natural occurrence of simple esters of **4a**, or of **4a** as a seed component, is previously unreported. Branched chain fatty acids, such as **3a**, are common in animal fats and in microorganisms but are rarely reported from higher plant sources [5, 6].

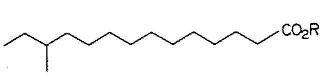


(**1a**) R=H
(**1b**) R=Ac



(**2a**) R=H
(**2b**) R=Me





(**3a**) R=H
(**3b**) R=Me

(**4a**) R=H
(**4b**) R=Ac

RESULTS AND DISCUSSION

Compound **1a** was isolated as a precipitate from a pentane–hexane extract of *S. plebeia* seed. An IR spectrum indicated the presence of OH and ester carbonyl functions. Prominent features in the MS of **1a** included an apparent M^+ at *m/e* 762 ($C_{45}H_{62}O_{10}$) and a base peak at *m/e* 137 ($C_8H_9O_2$). The PMR spectrum of the compound included a pair of strongly coupled doublets ($J = 16$ Hz) at δ 7.56 and 6.24, several aromatic proton signals, three OMe signals, a singlet attributed to a long $-CH_2-$ chain (δ 1.24) and a complex aliphatic Me signal at δ 0.85. Three OH groups were present in **1a** as indicated by D_2O exchange and by its conversion to a triacetate (**1b**).

Alkaline hydrolysis of **1a** yielded three products—**2a**, **3a** and **4a**—which were separated by preparative-TLC. Compound **2a** and its Me ester (**2b**) were identical in all respects (TLC, PMR, MS) with authentic samples of ferulic acid and Me ferulate. PMR and MS indicated that **3a** was a saturated fatty acid of MW 242 containing one Me branch. In addition, Me ester **3b** had the same GLC R_t as authentic Me 12-methyltetradecanoate on both Apiezon and Silar 5 CP columns and its MS was indistinguishable from one published for Me 12-methyltetradecanoate [7]. Compound **3a** was dextrorotatory, $[\alpha]_D + 5.1°$, confirming that it was (+)-12 L-methyltetradecanoic acid [6].

The structure of **4a** (M^+, *m/e* 362) and its acetylation product **4b** (M^+, *m/e* 530) were deduced by detailed PMR and MS analyses. The PMR of **4a** contained signals attributable to only half the expected number of protons, suggesting that it was a symmetrical dimer, and the following groups were assigned: aromatic –OMe (δ 3.75), $-CH_2O-R$ (δ 3.63), $Ar-CH_2-$ (δ 2.63) and $-CH<$ (δ 1.86). Upon acetylation, the signals at δ 3.63 shifted downfield to δ 4.12 and the shifted signals appear to be the AB portion of an ABX system. This was verified in a double resonance experiment where irradiation of the high field methine (X) proton collapsed the AB multiplet to an AB quartet. A PMR spectrum of dihydrocubebin, the methylenedioxy analog of **4a**, was essentially identical to that of **4a** except for expected differences due to the aromatic substituents [8]. The PMR data together with the MS establish that there are two equivalent $>CH-CH_2-O-R$ groups. A very prominent base peak, in the MS of **4a**, at *m/e* 137 ($C_8H_9O_2$) was good evidence for the presence of a OMe and OH disubstituted benzyl function [9]. The pattern observed for the aromatic PMR signals in **4a** placed the O functions at the *meta-para* positions and led to the conclusion that

4a could only be secoisolariciresinol. Compound 4a was levorotatory, $[\alpha]_D - 7°$, but considerably less so than the $[\alpha]_D - 30.8°$ reported for secoisolariciresinol [3]. It is probable that our material contained more than one stereoisomer. An authentic sample of 4a was not available for further comparison.

Thus, 1a is a ferulic acid–methyltetradecanoic acid diester of secoisolariciresinol, and the PMR is entirely consistent with this structure. Acetylation of 1a failed to produce a significant shift in the methylene multiplet at δ 4.28 demonstrating that the ester groups are at the 1 and 4 positions. The MS of 1a is also consistent with this structure, M^+, *m/e* 762 ($C_{45}H_{62}O_{10}$). Prominent ions at *m/e* 568 ($C_{35}H_{52}O_6$) and *m/e* 326 ($C_{20}H_{22}O_4$) must originate by consecutive losses of 2a and 3a. The ion at *m/e* 326 then corresponds to 4a minus 2 × H_2O, and the ion at *m/e* 177 ($C_{10}H_9O_3$) must arise from acyl-O cleavage of the ferulic acid group. The base peak in the spectrum of 1a, *m/e* 137 ($C_8H_9O_2$), is identical to that observed in the spectrum of 4a which is further evidence that the acids are not esterified at the aromatic positions. We have no basis for assigning stereochemistry at positions 2 and 3; however, the low rotation observed for 1a indicates that it is a mixture of unequal amounts of the two optically active (*threo*) isomers. The presence of an optically inactive *erythro* (*meso*) form is considered unlikely as diasteromers of 1a should be separable by GLC and by TLC on borated silica, whereas the two optically active *threo* forms would not be resolved under these conditions [10].

EXPERIMENTAL

Salvia plebeia seed was collected in India during 1974. The authors acknowledge receipt of the seed from Dr. Robert E. Perdue, Jr., Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Beltsville, MD. Mp's were determined on a Fisher–Johns block and are uncorrected. All compounds were analyzed by TLC on precoated Si gel F-254 plates. Compound 4a was also analyzed by TLC on a Si gel G plate prepared using a satd soln of boric acid. PMR spectra were recorded at 100 MHz in $CDCl_3$ solns with TMS as an internal standard, unless otherwise specified, and extensive decoupling was used to verify assignments. Low resolution MS were obtained by probe inlet at 70 eV and empirical formulae of major ions were obtained by high resolution. GLC analysis of the TMSi derivative of 1a was made on 1 m × 2 mm stainless-steel columns packed with 3% OV-1 programmed from 200–400° at 6°/min. Hydrolysis products were analyzed on 1 m × 2 mm glass columns packed with 5% Apiezon L or 3% Silar 5 CP operated at 180°.

Isolation and properties of 1a. Ground seed of *S. plebeia* (150 g) was Soxhlet extracted for 6 hr with 1 l. of pentane–hexane. The extract was cooled to room temp. and allowed to stand 18 hr. The white ppt which formed was recovered by filtering and washed repeatedly with small quantities of cold pentane–hexane yielding 1.05 g material (1a). Evaporation of the pentane–hexane soln gave 31 g glyceride oil. 1a gave a single spot when examined by TLC ($R_f = 0.44$; C_6H_6–$CHCl_3$–MeOH, 50:50:3) and a single peak when examined by GLC of its TMSi derivative; mp 78–80°; $[\alpha]_D^{26} - 26°$ ($CHCl_3$; *c* 1.10); IR ($CHCl_3$), ν_{max} 3650 cm^{-1} (–OH) and 1725 cm^{-1} (>C=O). The following PMR signals were observed for 1a: δ 7.56 (1H, *d*, $J = 16$ Hz), 7.20–6.40 (9H, m, aromatic), 6.24 (1H, *d*, $J = 16$ Hz), 5.97 (1H, *s*, –O$\underline{H}$), 5.53 (2H, *s*, –O$\underline{H}$), 4.28 (4H, *m*, –C$\underline{H}_2$–O–), 3.89 (3H, *s*, –OC$\underline{H}_3$), 3.74 (6H, *s*, –OC$\underline{H}_3$), 2.67 (4H, apparent *d*, Ar–C$\underline{H}_2$–CH), 2.25 (4H, *m*, –C$\underline{H}_2$–CO–O– and –C$\underline{H}$), 1.24 [*ca* 22H, *s*, –$(CH_2)_n$–], 0.85 (6H, *m*, –C$\underline{H}_3$ and CH–C$\underline{H}_3$). OH protons (δ 5.97 and δ 5.53) were readily exchanged in D_2O. MS of 1a (probe) 70 eV *m/e* (rel. int.): 762 M^+ (7), 568 (17), 326 (25), 189 (32), 177 (55) and 137 (100). Found: M^+, *m/e* 762.433; $C_{45}H_{62}O_{10}$ requires 762.434.

Preparation of 1b. A 75-mg portion of 1a was acetylated in HOAc–C_5H_5N (1:1) for 18 hr at 26°. Excess solvent was removed on a rotary evaporator, the residue dissolved in a $CHCl_3$–H_2O mixture and the aq layer was repeatedly extracted with $CHCl_3$. Combined $CHCl_3$ extracts gave 88 mg of 1b as a colorless glass which did not crystallize, $[\alpha]_D^{26} - 7°$ ($CHCl_3$; *c* 1.50); IR ($CHCl_3$), ν_{max} 1725 and 1765 cm^{-1} (double >C=O). A PMR spectrum of 1b is summarized as follows: δ 7.60 (1H, *d*, $J = 16$ Hz), 7.20–6.50 (9H, *m*, aromatic), 6.32 (1H, *d*, $J = 16$ Hz), 4.22 (4H, *m*, –C$\underline{H}_2$–O–), 3.82 (3H, *s*, –OC$\underline{H}_3$), 3.70 (6H, *s*, –OC$\underline{H}_3$), 2.73 (4H, apparent *d*, Ar–C$\underline{H}_2$–CH<), 2.27 (3H, *s*, C$\underline{H}_3$CO$_2$–), 2.24 (6H, *s*, C$\underline{H}_3$CO$_2$–), 1.24 (*ca*. 22H, –(C$\underline{H}_2$)$_n$–), 0.85 (6H, *m*, –Me and >CH–C$\underline{H}_3$). MS of 1b (probe) 70 eV *m/e* (rel. int.): 888 M^+ (1), 846 (17), 804 (10), 611 (16), 568 (20), 368 (8), 326 (30), 219 (9), 189 (40), 177 (62) and 137 (100).

Saponification of 1a *and isolation of products*. 1a (100 mg) was saponified by refluxing for 2 hr in a soln of N aq KOH (10 ml) and MeOH (10 ml). The soln was conc. under N_2 at 100° and adjusted to pH 6 by addition of HOAc. H_2O was then added and the resulting soln repeatedly extracted with 10-ml portions of $CHCl_3$. Combined $CHCl_3$ extracts yielded 107 mg of a mixture of 3 products observed by TLC and GLC. Preparative-TLC of the mixture gave 2a (14 mg, $R_f = 0.52$), 3a (30 mg, $R_f = 0.72$) and 4a (43 mg, $R_f = 0.57$) using a C_6H_6–$CHCl_3$–MeOH (5:5:2) solvent system. Under the same conditions, 1a had an R_f of 0.74 and authentic ferulic acid (2a) gave an R_f of 0.52. 4a gave 1-spot on a boric acid impregnated plate, $R_f = 0.65$.

Ferulic acid (2a). 2a and authentic ferulic acid gave identical PMR spectra, in $(CD_3)_2O$, and the MS of both were indistinguishable. A portion of 2a was esterified (H_2SO_4–MeOH) yielding Me ester 2b: MS (probe) 70 eV *m/e* (rel. int.): 208 M^+ (100), 177 (63), 145 (27), 134 (8), 133 (10), 117 (11), 105 (6) and 89 (10).

12-L-Methyltetradecanoic acid (3a). 3a was isolated as a colorless oil, $[\alpha]_D^{26} + 5.1°$ ($CHCl_3$; *c* 0.26), and gave an IR spectrum consistent with that expected for a fatty acid. PMR of 3a: δ 2.26 (2H, *t*, –C$\underline{H}_2$–CO$_2$H), 1.58 (2H, *m*), 1.26 (*ca* 21H, *s*, –(C$\underline{H}_2$)$_n$–), 0.85 (6H, *m*, –C$\underline{H}_3$ and >CH–C$\underline{H}_3$). MS of 3a (probe) 70 eV *m/e* (rel. int.): 242 M^+ (26), 213 (11), 199 (11), 195 (17), 185 (39), 177 (13), 129 (31), 111 (21), 97 (36), 85 (25), 83 (30), 73 (49), 71 (35), 69 (35), 60 (46), 57 (100), 55 (63), 43 (47) and 41 (37). A portion of 3a was esterified (H_2SO_4–MeOH) yielding Me ester 3b: MS (GLC) 70 eV *m/e* (rel. int.): 256 M^+ (13), 227 (5), 225 (4), 213 (9), 199 (12), 143 (15), 97 (15), 87 (72), 83 (15), 74 (100), 69 (20), 57 (41), 55 (44), 43 (32) and 41 (27).

Secoisolariciresinol (4a). 4a was obtained as a white solid, $[\alpha]_D^{26} - 7°$ (Me_2CO; *c* 0.12), containing 2a as a minor impurity (TLC and PMR). PMR of 4a ($CDCl_3$–CD_3OD): δ 7.10–6.40 (6H, ABC multiplet typical of a trisubstituted aromatic ring) [11], 3.75 (6H, *s*, –OC$\underline{H}_3$), 3.63 (4H, *m*, –CH$_2$–O), 2.63 (4H, broad *d*, Ar–C$\underline{H}_2$–) and 1.86 (2H, *m*, –CH).* A probe MS of 4a first revealed a spectrum attributed to the impurity (2a). By increasing the probe temp, 2a was removed until only the spectrum of 4a remained: MS (probe) 70 eV *m/e* (rel. int.): 362 M^+ (28), 344 (5), 189 (11), 163 (6), 150 (8), 138 (32), 137 (100) and 122 (9).

Secoisolariciresinol tetraacetate (4b). 4a (36 mg) was acetylated in a manner identical to that used for acetylation of 1a and the crude product, 41 mg, was purified by preparative-TLC in C_6H_6–$CHCl_3$–MeOH (50:50:3). 4b (34 mg) was obtained as a colorless glass $[\alpha]_D^{26} - 7.6°$ ($CHCl_3$; *c* 0.65); IR ($CHCl_3$), ν_{max} 1740 and 1775 cm^{-1} (double >C=O). PMR of 4b: δ 6.96–6.50 (6H, *m*, aromatic ABC multiplet), 4.12 (4H,

* As 4a and 4b are symmetrical dimers, integration of their PMR spectra gave only half the number of protons reported here.

m, $-CH_2-O-$), 3.71 (6H, *s*, $-OCH_3$), 2.67 (4H, apparent *d*, $Ar-CH_2-$), 2.25 (6H, *s*, aromatic, CH_3CO_2-), 2.17 (2H, *m*, $-CH$) and 2.02 (6H, *s*, aliphatic CH_3CO_2-).* MS of **4b** (probe) 70 eV *m/e* (rel. int.): 530 M^+ (4), 488 (33), 446 (30), 386 (9), 189 (20), 138 (21), 137 (100) and 43 (44).

Acknowledgements—We are indebted to Dr. D. Weisleder for PMR spectra, to Dr. W. K. Rohwedder for high resolution MS, to Dr. P. L. Schiff, Jr. for a gift of dihydrocubebin, and to R. V. Madrigal for technical assistance. Positive identification of *Salvia plebeia* was made by Sandra Saufferer, Medicinal Plant Resources Laboratory, USDA, Beltsville, Md.

REFERENCES

1. Briggs, L. H., Cambie, R. C. and Hoare, J. L. (1959) *Tetrahedron* **7**, 262.
2. Freudenberg, K. and Weinges, K. (1959) *Tetrahedron Letters* No. 17, 19.
3. Erdtman, H. and Tsuno, K. (1969) *Acta Chem. Scand.* **23**, 2021.
4. Erdtman, H. and Tsuno, K. (1969) *Phytochemistry* **8**, 931.
5. Polgar, N. (1971) *Top. Lipid Chem.* (Gunstone, F. D., ed.) Vol. 2, pp. 210–211. Wiley, New York.
6. Smith, C. R., Jr. (1970) *Top. Lipid Chem.* (Gunstone, F. D., ed.) Vol. 1, pp. 282–285. Logos Press, London.
7. Tyrrell, D. (1968) *Lipids* **3**, 368.
8. Duffield, A. M. (1967) *J. Heterocycl. Chem.* **4**, 16.
9. Dwuma-Badu, D., Ayim, J. S. K., Dabra, T. T., ElSohly, H. N., Knapp, J. E., Slatkin, D. J. and Schiff, P. L., Jr. (1975) *Lloydia* **38**, 343.
10. Morris, L. J. (1963) *J. Chromatog.* **12**, 321.
11. Bovey, F. A. (1969) *Nuclear Magnetic Resonance*, p. 113. Academic Press, New York.